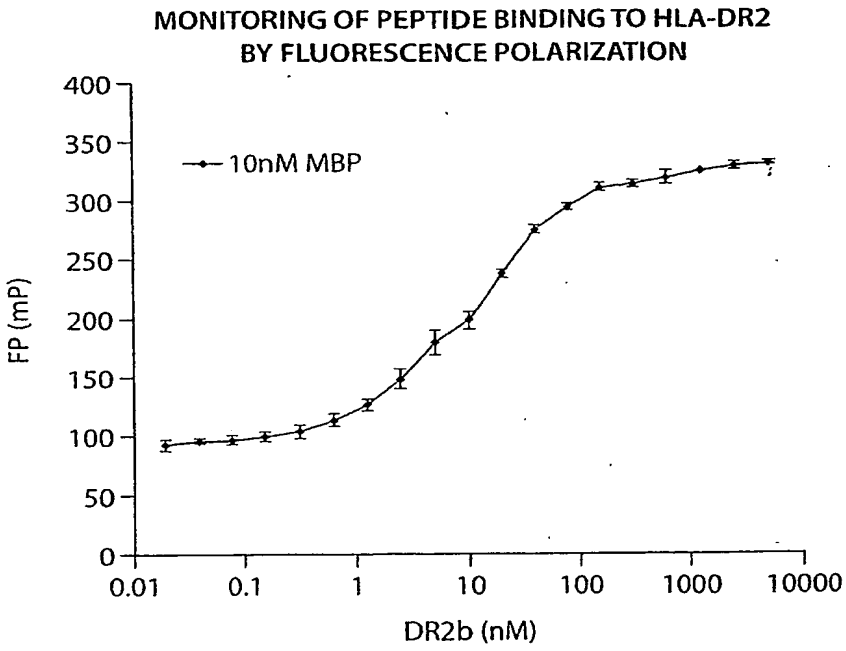

MONITORING OF PEPTIDE BINDING TO HLA-DR2
BY FLUORESCENCE POLARIZATION
10nM MBP
FP (mP)
400
350
300
250
200
150
100
50
0
0.01
0.1
1
10
100
1000
10000
DR2b (nM)

Fig. 1

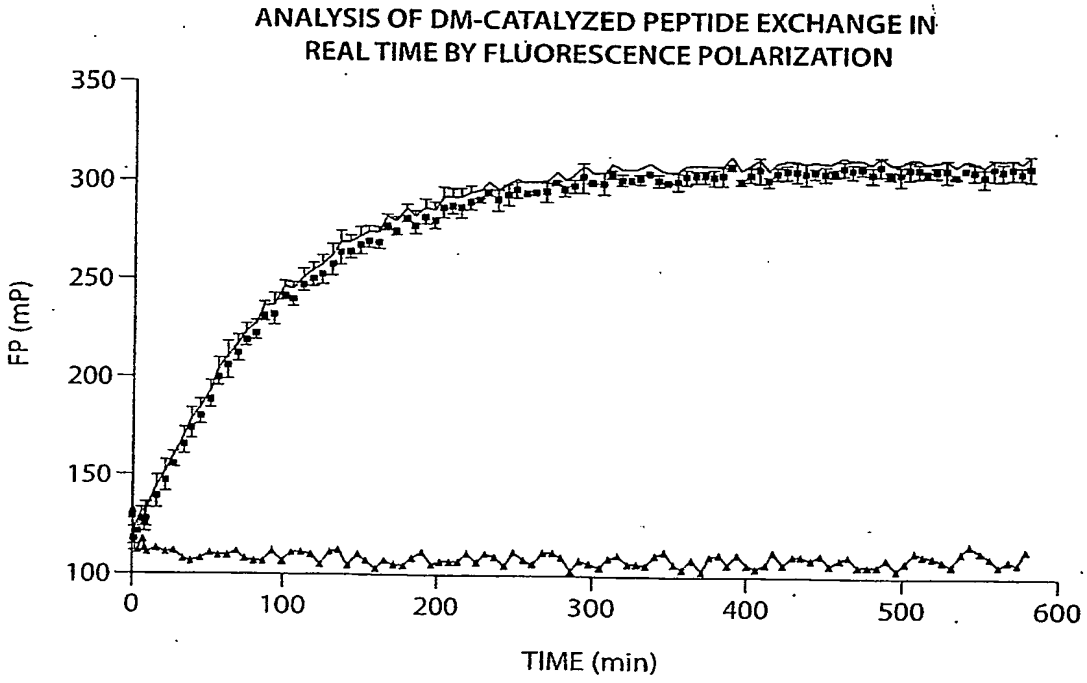

ANALYSIS OF DM-CATALYZED PEPTIDE EXCHANGE IN REAL TIME BY FLUORESCENCE POLARIZATION
FP (mP)
350
300
250
200
150
100
0
100
200
300
400
500
600
TIME (min)

Fig. 2

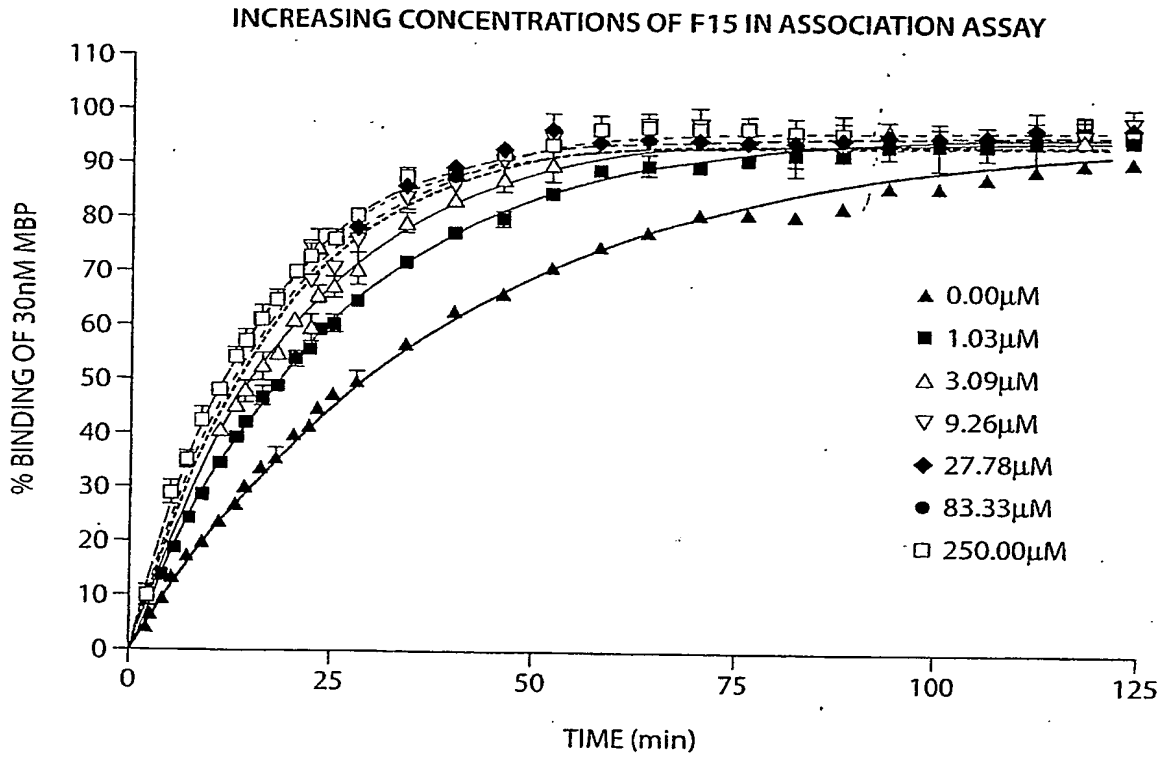
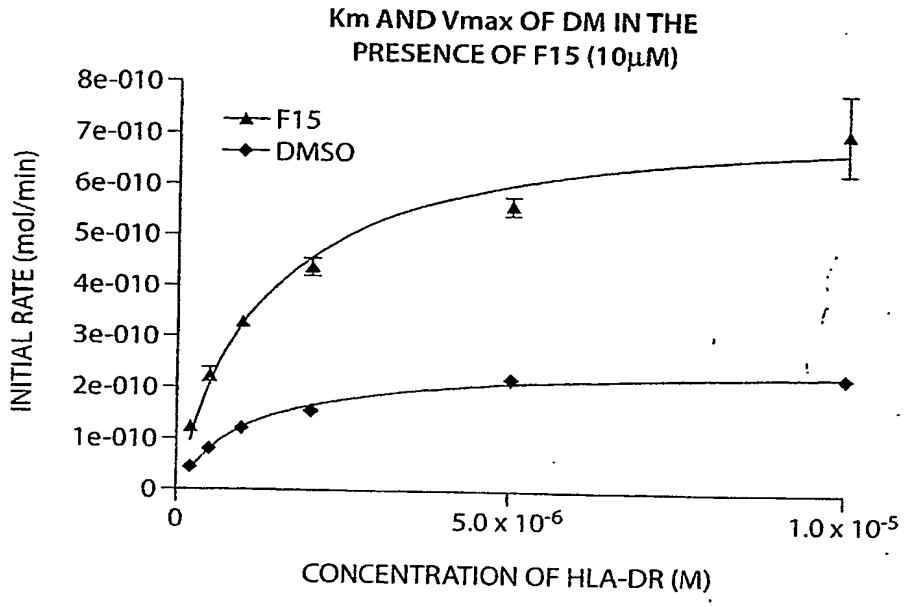

INCREASING CONCENTRATIONS OF F15 IN ASSOCIATION ASSAY
% BINDING OF 30nM MBP
TIME (min)
0
10
20
30
40
50
60
70
80
90
100
110
0
25
50
75
100
125
0.00µM
1.03µM
3.09µM
9.26µM
27.78µM
83.33µM
250.00µM

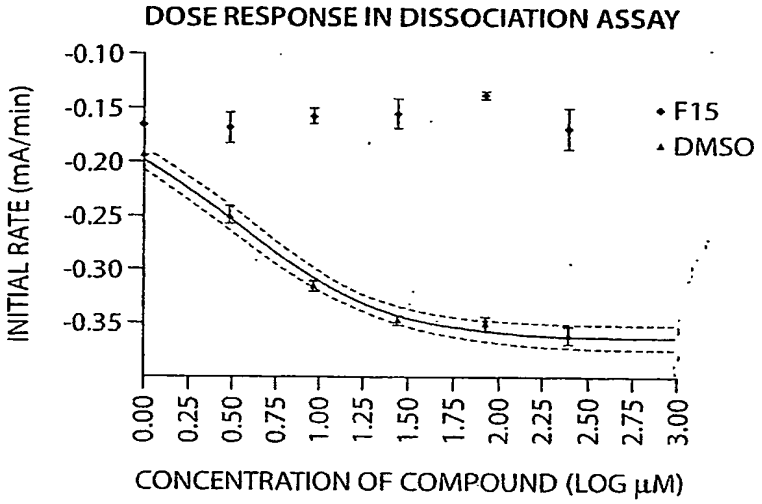
Fig. 3



CALCULATE Km AND Vmax FROM INITIAL RATES

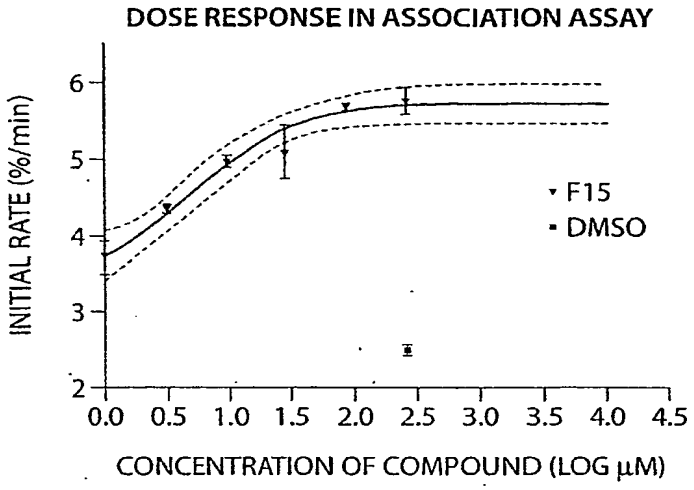
BEST-FIT VALUES	F15	DMSO
VMAX ($x10^{-8}$)	0.07611	0.02521
PEP	1.000e-008	1.000e-008
KM	1.343e-006	1.055e-006
Std. ERROR		
VMAX	0.004248	0.0005050
KM	2.340e-007	7.033e-008
95% CONFIDENCE INTERVALS		
VMAX	0.06710 TO 0.08512	0.02414 TO 0.02628
KM	8.465e-007 TO 1.839e-006	9.059e-007 TO 1.204e-006
GOODNESS OF FIT		
DEGREES OF FREEDOM	16	16
R^2	0.9269	0.9875

Fig. 4



	F15
SIGMOIDAL DOSE-RESPONSE	
BEST-FIT VALUES	
BOTTOM	-0.3653
TOP	-0.1478
LOGEC50	0.5105
EC50	3.239
STD. ERROR	
BOTTOM	0.005449
TOP	0.008180
LOGEC50	0.07086
95% CONFIDENCE INTERVALS	
BOTTOM	-0.3768 TO -0.3539
TOP	-0.1650 TO -0.1306
LOGEC50	0.3616 TO 0.6593
EC50	2.299 TO 4.564
GOODNESS OF FIT	
DEGREES OF FREEDOM	18
R^2	0.9727

Fig. 5a



	F15
SIGMOIDAL DOSE-RESPONSE BEST-FIT VALUES	
BOTTOM	3.278
TOP	5.745
LOGEC50	0.6590
EC50	4.560
STD. ERROR	
BOTTOM	0.3276
TOP	0.1249
LOGEC50	0.1912
95% CONFIDENCE INTERVALS	
BOTTOM	2.575 TO 3.981
TOP	5.477 TO 6.012
LOGEC50	0.2488 TO 1.069
EC50	1.774 TO 11.73
GOODNESS OF FIT	
DEGREES OF FREEDOM	14
R^2	0.8980

Fig. 5b

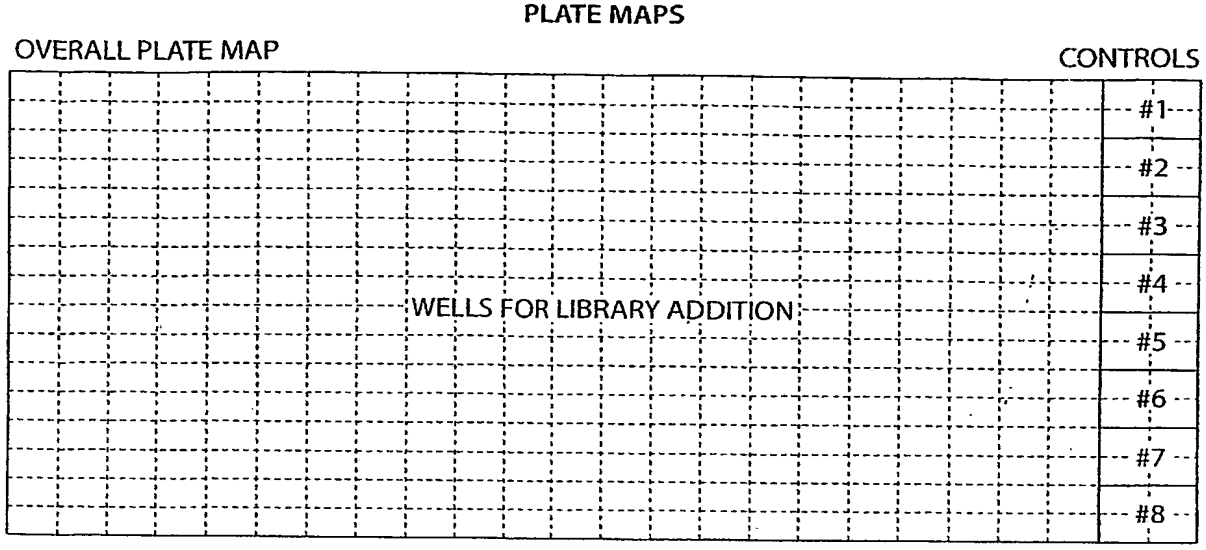


PLATE MAPS
OVERALL PLATE MAP
CONTROLS
#1
#2
#3
#4
WELLS FOR LIBRARY ADDITION
#5
#6
#7
#8

Fig. 6

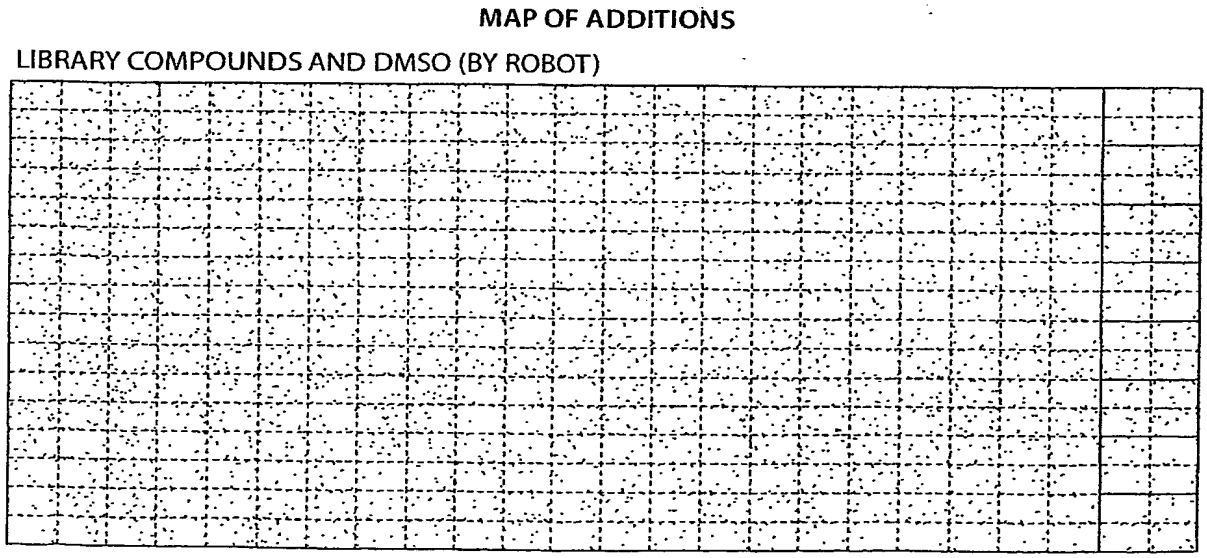

MAP OF ADDITIONS
LIBRARY COMPOUNDS AND DMSO (BY ROBOT)

Fig. 7

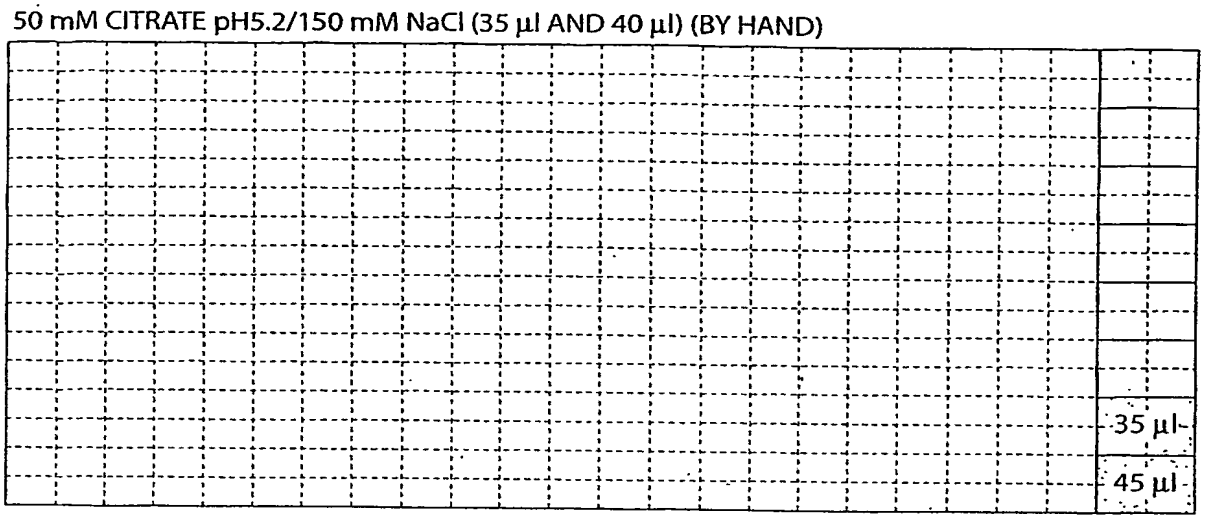

50 mM CITRATE pH5.2/150 mM NaCl (35 µl AND 40 µl) (BY HAND)
35 µl
45 µl

Fig. 8

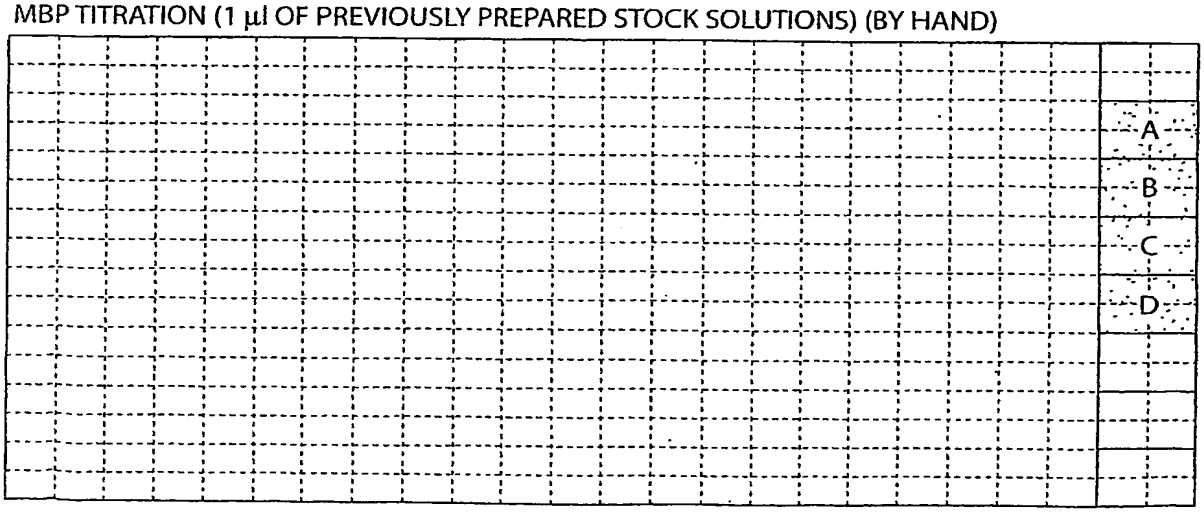

MBP TITRATION (1 µl OF PREVIOUSLY PREPARED STOCK SOLUTIONS) (BY HAND)
A
B
C
D

Fig. 9

35 µl HLA-DR2b (BY ROBOT)

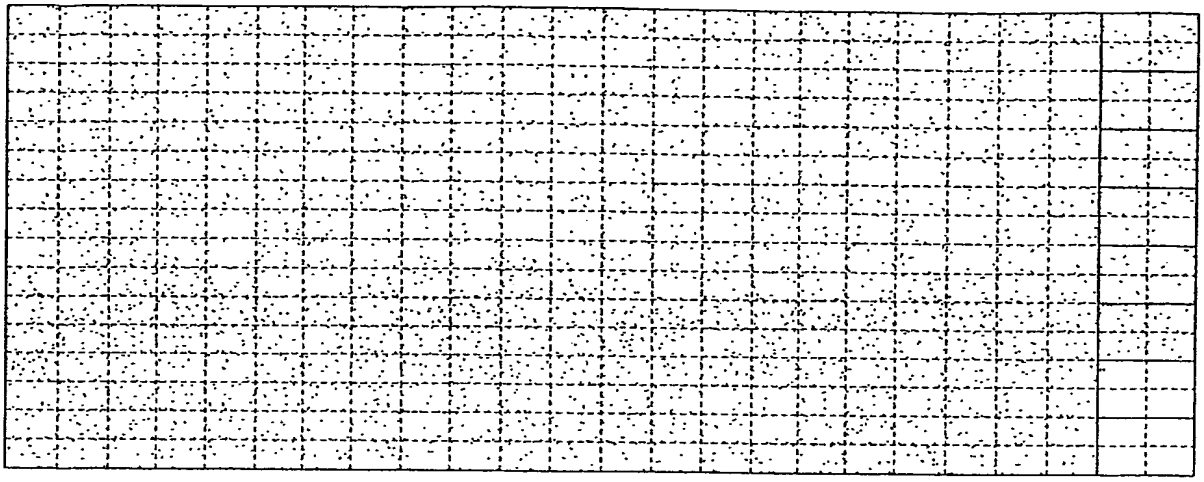


Fig. 10

5 µl OF 80 nM MBP (ALEXA 488 AT P5) (BY HAND)

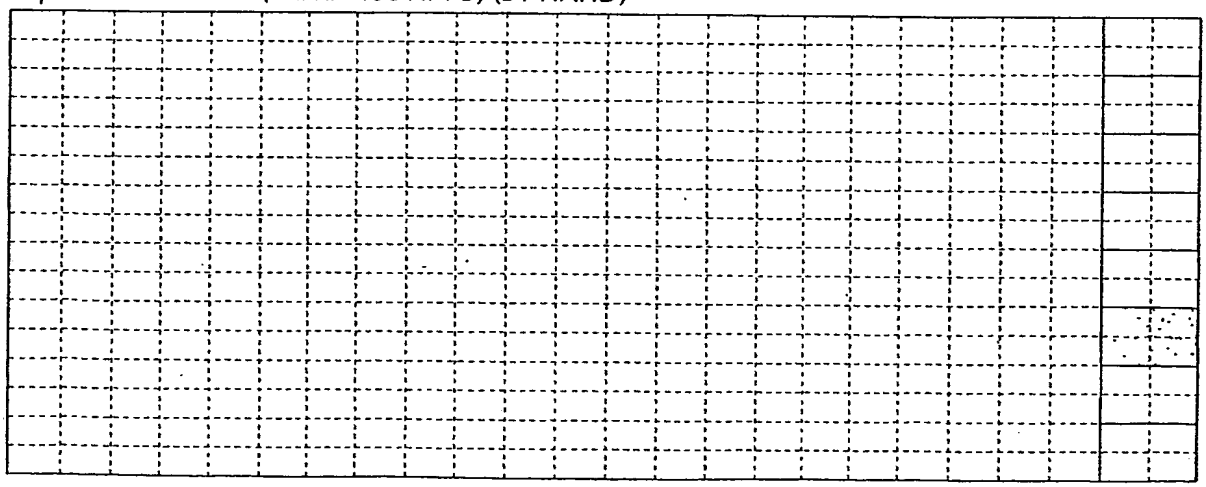


Fig. 11

5 µl HLA-DM/MBP ADDITION (BY ROBOT)

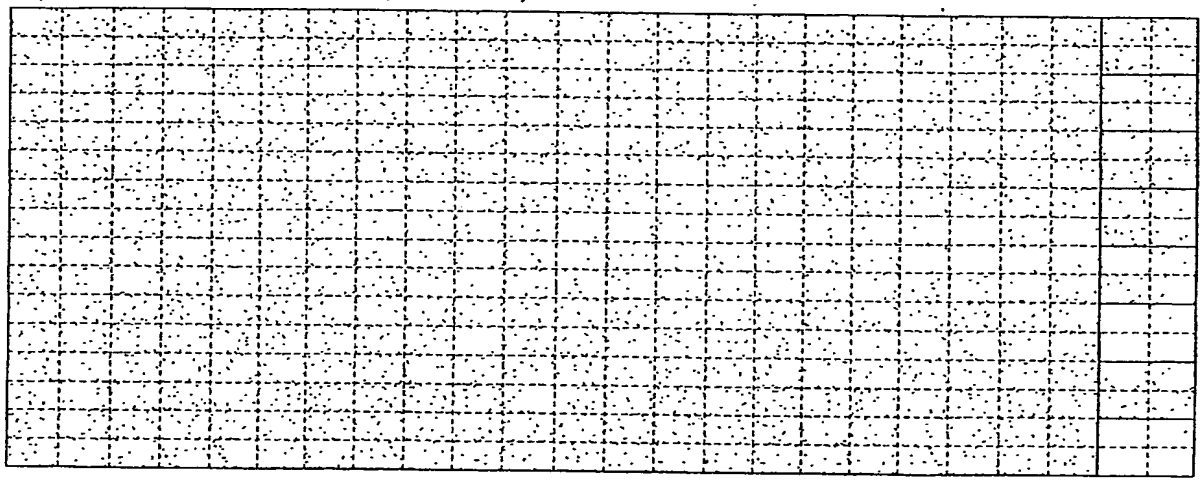


Fig. 12